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COMPARISON OF AROMA COMPOUNDS IN DISTILLED AND EXTRACTED PRODUCTS OF SAGE (*SALVIA OFFICINALIS L.*)

SUMMARY

Salvia officinalis, a member of *Lamiaceae* family, is a valuable medicinal and aromatic plant. From its Latin name, ‘‘Salvia’’ meaning to cure and ‘‘Officinalis’’meaning medicinal, it is clear that sage has a historical reputation for promotion of health and treatment of ailments. Modern day research has shown that sage essential oil can improve the memory and has shown promise in the treatment of Alzheimer’s disease. It has been used since ancient times as a local anesthetic, for nasal discharge associated with upper respiratory infections and for the skin. *Salvia officinalis* is growing in many areas of Europe and also used for distillation, in order to produce essential oils with therapeutic properties. Dioscorides, Pliny and Galen all recommended *Salvia officinalis* as a haemostatic, tonic and for hyperlipidemia.

Although extraction product likes concrete, absolute and hydrosol from the oil of sage, have an economical interest in food industries and there are very limited studies on these products. Sage oil and sage water (hydrosol) were released from the herb of sage plants by using steam distillation. Sage concrete was extracted also from the herb by using pentane and diethyl ether (50:50) as a solvents and sage absolute was produced from the concrete after ethyl alcohol extraction. Essential oil from these products were analyzed by GC-MS. 1.8-cineole; Camphene and Camphor, were the major component of sage essential oil, hydrosol oil, sage concrete and sage absolute. Small concentrations in thujones, toxic compounds which can cause absinthism, were found in the studied *Salvia officinalis* L. samples.

Key words: Sage, *Salvia officinalis* L., Essential oil.

INTRODUCTION

Salvia officinalis L. (Lamiaceae) is a plant family with approximately 900 species widespread through the world. In Flora Europea 36 taxa are described. As its Latin gender name Salvia means “to cure” and species name “officinalis” means medicinal, it is clear that sage has a historical reputation of promoting health and treating ailments .In Ancient Rome, it was even called the sacred plant

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(Baydar et al,. 2013). The genus *Salvia* L. is well known as a common antibacterial, hypoglycemic, antiflammatory, fungistatic, virustatic, astringent, eupectic ,antihydrotic, and anti-diabetic products which is used in food preservation or for medical and pharmaceutical care (Hohmann et al., 2003). *Salvia officinalis* is endemic in many areas of Greece.Many compounds belonging to the groups of terpenoids, flavonoids, anthocyanins, phenolics acids, phenolics glycosides with strong antioxidant and antiradical activities and also antibacterial properties (Lu et al., 2002; Kintzios, 2000). The extract of *Salvia officinalis* species were exhibited excellent antioxidant activities when compared to butylated hydroxytoluene(BHT), it seems possible to keep perishable fat – containing food longer by direct addition of an extract of *Salvia officinalis* (sage). Distillates of Sage, an hydrosol, is released from a by-product during distillation can used as natural beverage with natural antibiotic against digestion disorders. These Hydrosols contain essential oil in the distillation water and has a very pleasant aroma with many commercial applications. Concrete of sage are extracted from the plant materials and volatile solvents such pentane and diethyl-ether (50:50). After the extraction the solvents are evaporated and the residues, called concretes, used in pharmaceutical cosmetics applications. The concrete contains aromatic compounds like terpenes, sesquiterpenes but also fatty acids, paraffin and other high molecular weight compounds (Miladinovic et al., 2000). In the aroma production industries, they used wax-free residues, after extraction of the concrete with ethanol. The ethanol extraction cooled to about -25^0 C, filtered to eliminate waxes and then concentrated by vacuum distillation in order to have more concentrated aromatic compounds. This study was aimed to investigate the comparison of aroma compounds of distillation and extraction products (Wang et al., 1998) from Greek Herb of *Salvia officinalis* L.(sage).

MATERIAL AND METHODS

Plant material: *Salvia officinalis* L. plants (it a study that began many years ago and it continues until to now) in three different micro- environments, from the Aegean Islands of Limnos and Saint Stratis, areas with typical Mediterranean climate. The samples were transported in polyoropylene bags to the laboratory and held at room temperature until air dried.

Extraction Essential oil and Hydrosol production: The essential oils in the fresh herb were extracted by steam distillation in a pilot distillation apparatus in Agricultural University of Athens. For each experiment, 1Kg of air dried *Salvia officinalis* L.(sage) herb was used into the distillation apparatus . The water stream with the essential oil was carried to a water –cooled condenser where the steam is condensed[1]. The essential oil and the distillate water (hydrosol) were separated in a Florentine flask and stored in vials.

Concrete and absolute production: The milled plant material (50gr) was added to an Erlenmeyer flask and extracted with pentane and diethyl ether(50:50) for 45min at 40^0C.Then the solvents was evaporated from the extract by rotary evaporator. A waxy, green semi-solid material (concrete) was obtained. The concrete was dissolved in analytical grade-ethyl alcohol (Merck 96%) and the alcoholic solution was cooled at 20^0C in a deep freezer under vacuum and finally left to stand overnight in a deep freezer and evaporated in vacuum using rotary evaporator to yield to absolute. The *Salvia officinalis* L. (sage) concrete and absolute were stored at 4^0C until GC-MS analyses.

Chemical analysis of volatile compounds by head space CG/MS: The volatile components present in headspace fraction of distillates were isolated and identified by using a balance pressure headspace system Perkin-Elmer HS40 (Perkin-Elmer Analytical Instruments, Uberlingen, Germany) coupled to a GC/MS-Q 5050 system (Shimadzu Co, Kyoto, Japan). A 2ml sample from each samples was taken and introduced into a 22 mL round-bottomed vial with 1 mL aliquot of standard solution {2mg pentanol – 3 in ethanol-water solution(50+50 v/v)}; then, the vials were sealed with aluminium-rubber septa. The vials with samples were held at 50°C for 25 min, purged and pressurised with helium at a flow rate of 40 mL/min. The volatile compounds were driven through the transfer line which was held at 80° C to the injector of the Gas Chromatograph. The volatile compounds were separated on an HP Innowax capillary column (60 m length×0.25 mm internal diameter, 0.25 μm film thickness) at the following conditions: injector temperature 200 °C; carrier gas helium 0.6 ml/min; temperature program: 45–100 °C at a rate of 4 °C min^{-1}, held for 5 min and go to 200 °C at a rate of 8 °C min^{-1} and held for 12min. The GC column was directly connected without splitting to the ion source of QP 5050 quadrupole mass spectrometric detector which was operating in the scan mode within a mass range of *m/z* 30–350 at 2 scans/s. The interface line to MS was set at 250 °C. The MS was operating in an electron impact mode at electron energy of 70 eV and was calibrated by autotuning. Identification of the compounds was carried out by computer-matching of their mass spectral data with those of known compounds in the Shimadzu NIST62 Mass spectral Database and by comparing their retention times and mass spectra to 3-pentanol as internal standards solution. Quantification was performed by integrating the peak areas of total ion chromatograms (TIC) by the Shimadzu Class 500 software. Oven temperature programme, 50–260 °C at a rate of 4 °C/min; transfer line temperature, 270 °C; carrier gas, helium at a linear velocity of 31.5 cm/s; inlet split ratio, 1:60; MS source ionization energy, 70 eV; scan time, was 1 s, covering a mass range of 40–300 amu. The constituents were identified by comparison of their mass spectra with those in a computer library (LIBR-TR and Wiley 5 Library) or with authentic compounds. The identifications were confirmed by comparison of their retention indices of volatiles either with those of authentic compounds or with

data in the literature. Quantitative results were obtained by calculating the average value of three samples.

RESULTS AND DISCUSSION

The essential oil composition of *Salvia officinalis* L. is known for its remarkable variability in aromatic qualities and bioactivity. I this study major compounds (%) in the studied distilled and extracted of salvia officinalis L.(sage) samples are listed in Table 1 and Table 2.Identified 19 different compounds.

Table 1.The major compounds identified (%)in salvia officinalis L. samples from the Aegean Island of Limnos

Component	RI*	Essential oil	Hydrosol	Concrete	Absolute
a-pinene	7.45	2.67	2.3	2.2	4.6
Camphene	7.97	19.78	21.33	20.29	8.16
β-pinene	8.94	3.56	2.1	tr	tr
Myrcene	9.35	1.65	1.12	2.06	tr
p-Cymene	10.46	0.11	tr	tr	tr
Limonene	10.67	2.14	1.05	1.45	1.77
γ-Terpinene	11.89	2.23	1.54	1.59	0.89
p-cymene	10.46	0.29	tr	tr	tr
1.8-Cineole	12.67	18.13	20.16	19.16	3.18
β-Thujone	13.56	4.5	4.3	3.4	3.2
α-Thujone	14.11	tr	tr	tr	tr
Camphor	14.81	24.87	40.65	25.14	15.27
Borneol	15.56	tr	tr	tr	Tr
α-Terpineol	16.79	0.9	tr	tr	0.17
Myrtenol	16.86	0.91	tr	tr	0.79
Verbenone	17.68	0.23	0.32	tr	0.82
Caryophyllene	21.55	0.19	0.24	2.09	3.16
Cis-α-bergamotene	23.71	0.19	tr	tr	Tr
Manool	26.71	tr	tr	tr	Tr
Phyllocladene	29.81	4.68	3.23	4.16	4.11
a-gurjunene	29.97	0.98	0.81	0.70	0.77

*(RI),Retention index,tr trace,(<0.01%),% realtive percentage

Table 2.The major compounds identified (%) in salvia officinalis L. samples from the Aegean Island of Saint Stratis.

Component	RI	Essential oil	Hydrosol	Concrete	Absolute
a-pinene	7.45	3.22	0.5	0.4	0.7
Camphene	7.97	19.61	23.56	21.33	8.64
β-pinene	8.94	2.91	2.11	tr	tr
Myrcene	9.35	4.11	3.42	1.11	tr
p-Cymene	10.46	0.23	tr	tr	tr
Limonene	10.67	2.67	1.12	1.56	1.98
γ-Terpinene	11.89	1.31	0.67	0.56	0.91
p-cymene	10.46	0.34	tr	tr	tr
1. 8-Cineole	12.67	19.23	21.56	20.13	4.78
β-Thujone	13.56	5.6	4.2	3.6	5.8
α-Thujone	14.11	0.5	0.4	tr	tr
Camphor	14.81	16.87	30.76	26.75	16.11
Borneol	15.56	2.56	4.87	4.98	3.11
α-Terpineol	16.79	0.13	tr	tr	0.16
Myrtenol	16.86	0.23	tr	tr	0.31
Verbenone	17.68	0.34	0.36	tr	0.89
Caryophyllene oxide	21.55	0.23	0.27	2.11	3.99
Cis-α-bergamotene	23.71	0.19	0.16	tr	0.45
Manool	26.71	0.13	0.16	tr	0.19
Phyllocladene	29.81	5.11	4.83	4.46	4.99
a-gurjunene	29.97	0.86	0.82	0.77	0.79

*(RI),Retention index, tr -trace,(<0.01%),% relative percentage

The samples from the Aegean island of Limnos are listed in table 1 and the compounds with the higher concentration are:1.8-Cineole has 18.13% in essential oil, 20.16% in hydrosol, 19.16% in concrete and 3.18% in absolute. Camphene has 19.78% in essential oil, 21.33% in hydrosol, 20.29% in concrete and 8.16% in absolute, Camphor has 24.87% in essential oil, 40.65% in hydrosol, 25.14% in concrete and 15.27% in absolute product. α-pinene is 2.67% in essential oil,2.3% in hydrosol 2.2% in concrete and 4.6% in absolute. The compounds with the less concentration in the samples from the area of Limnos is Manool, Borneol and α-thujone with traces (<0.01%) in essential oil, in hydrosol, in concretes and in absolute samples respectively. The samples from the Aegean island of Saint Stratis are listed in table 2 and the compounds with the higher concentration are: 1.8-Cineole has 19.23% in essential oil, 21.56% in hydrosol,20.13% in concrete and 4.78 in absolute. Camphene has 19.61% in

essential oil, 23.56% in hydrosol, 21.33% in concrete and 8.64% in absolute, Camphor has 16.87% in essential oil, 30.76% in hydrosol, 26.75% in concrete and 16.11% in absolute product. α-pinene is 3.22% in essential oil,0.5% in hydrosol 0.4% in concrete and 0.7% in absolute. The compounds with the less concentration in the samples from the area of Limnos is α-terpineol with 0.13% in essential oil and traces in hydrosole, concrete and absolute, Manool with 0.13% in essential oil,0.16% in hydrosole, traces inconcrete and 0.19% in absolute in the studied samples respectively. Comparison between the Salvia officinalis L. from other countries the toxic thujones (α- and β- thujone) in the samples from Estonia was 28.1- 36.9% respectively and from Ukraine,Scotland, and Belgium 3.4-14.2% from Russia (23.3%) and Hungary(25.2%)[16,17,18]. Routine analysis of *Salvia officinalis* L. from different geographical areas to distinguish the origin, it still needs to be clarified.The present study showed that *Salvia officinalis* L.(sage) is a valuable aromatic plant especially in food industries. Also thujones a toxic compound has very low concentration in comparison with other geographical areas.

CONCLUSIONS

The analysis of extracted and distilled Salvia officinalis L. (sage) samples from the geographical areas of Aegean Sea gives potential and beneficial results especially for oil crop distilleries, producing marketable aromatic materials with high quality. This research is the first step, in the distillation and extraction of valuable aromatic plants from those geographical areas and will be needs more extended research with samples of salvia officinalis L.(sage) from other geographical areas.

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